2013. The Journal of Arachnology 41:53–58

Variation among clutches in the response of spiders to prey nutrient content

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Abstract. The phenotype of animals is often determined by an interaction between genes and the environment. In spiders, recent work has shown that the nutritional composition of prey can have a large effect on the growth and reproduction of spiders. I tested whether the growth of juvenile spiderlings was affected by an interaction between the clutch and the diet on which they fed (i.e., high or low nutrient) in both a wandering (*Tigrosa helluo* (Walekenaer 1837)) and a web-building (*Pholcus phalangioides* Fuesslin 1775) spider. Diet was manipulated by feeding spiderlings similar quantities of food that varied in their nutritional eomposition. The results for both species followed the same pattern. Overall, spiderlings fed the high-nutrient prey were larger, both in terms of mass and body size. However, there was significant variation in effect size among clutches, with some clutches showing large effects of nutrients on growth and other clutches showing little or no effect of nutrients on growth. In both species, there were no differences among clutches in the final mass and size of individuals on the low nutrient treatment. The differences among clutches were due to differences in the mass and size of spiderlings on the high nutrient treatments. These results highlight the importance of incorporating a diverse range of clutches or genotypes in studies of spider nutrition to ensure that the results are generalizable and not biased by particular genotypes or clutches.

Keywords: Diet, *Pholcus phalangioides*, *Tigrosa helluo*

The quantity and nutrient content of food available in nature is a major factor affecting the survival, growth and reproduction of spiders (Wise 1993, 2006). In particular, the addition of lipid, protein, or a eombination of nutrients to prey ean affect the life history and behavior of a range of wandering and web-building spiders (Mayntz & Toft 2001; Jespersen & Toft 2003; Mayntz & Toft 2006; Wilder 2011). Arthropod prey can vary widely in their nutrient content in nature, and understanding how nutrients affect spider performance can better aid in predicting prey choice by spiders or the potential consequences of ehanges in prey communities for spider populations.

The clutch that a spider belongs to can also have significant effects on survival and growth (Jakob & Dingle 1990; Uhl et al. 2004). Spiders from some clutches grow faster or larger than those from other clutches due either to maternal or genetic effects on offspring growth (Jakob & Dingle 1990; Uhl et al. 2004). Clutch can also interact with food quantity to affect spider growth, resulting in some clutches showing a greater response to increases in food availability than others (Jakob & Dingle 1990; Balfour 2004; Uhl et al. 2004). For example, in pholcid spiders (Araneae: Pholcidae), body mass, development time and body size are heritable, and this heritability contributes to signifieant gene by environment interactions in the growth of spiderlings (Jakob & Dingle 1990; Uhl et al. 2004). However, it remains unknown whether clutch and prey nutrient content also interact to affect spider survival or growth. Testing for clutch by diet interactions is important because it affects the selection of animals for experiments. If there are significant interactions between clutch and diet, then care would need to be taken to include a diverse range of clutches or genotypes of spiders in experiments to ensure that the results are generalizable and not biased by particular genotypes or clutches.

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The purpose of this study was to test whether clutches of spiders differed in the effects of prey nutrient content on growth. Using a split-clutch design, I compared growth rates of several clutches of wandering and web-building spiders fed high or low nutrient prey (*Drosophila melanogaster* that had either been raised on standard or nutrient supplemented media; Mayntz & Toft 2001). This experiment was conducted using clutches from three females of the wandering spider *Tigrosa* (formerly *Hogna*) *helluo* (Walkenaer 1837) (Araneae: Lycosidae) and four females of the web-building spider *Pholcus phalangioides* Fuesslin 1775 (Araneae: Pholcidae). These species were not directly compared, but were used to test whether clutch effects and interactions of clutch and food quality occurred in spiders with different life history strategies. Significant differences in growth between clutches or qualitative differences in the way that clutches responded to diet treatments would indicate that clutch is an important factor to include in future studies of spider growth.

METHODS

Drosophila melanogaster were used as prey in these experiments. Diptera may be an important component in the diet of both wandering (e.g., Nyffeler & Benz 1988) and web-building spiders (e.g., Jmhasly & Nentwig 1995), and the nutritional content of Diptera can vary substantially in the field (Markow et al. 1999; Jaenike & Markow 2003). In the laboratory, the nutritional quality of individuals of a single species of *Drosophila* spp. can be manipulated through the composition of the media on which they are raised (Markow et al. 1999; Mayntz & Toft 2001; Jaenike & Markow 2003; Mayntz et al. 2003; Jespersen and Toft 2003; Mayntz et al. 2005; Mayntz and Toft 2006). For the purposes of this study, vestigial-winged *D. melanogaster* were raised on either potato flake medium (Ward's^{TM} Instant *Drosophila* Medium) or potato flake medium supplemented with 40% dog food (Ol' Roy^{TM} Dog Food; 21% protein) by mass. There were no differences in the dry mass or percent C of flies raised on these treatments, but flies reared on the dog-food-supplemented

Table 1.—Results of repeated measures analysis of variance for the effects of prey nutrient content (low or high) and clutch on the mass of juvenile *Tigrosa helluo* and *Pholcus phalangioides* over time.

	Tigrosa helluo				*Pholcus phalangiodes*			
Source of Variation	df	MS	F	P	df	MS	F	P
Treatment	1	163.3	25.8	< 0.001	1	12.9	9.2	< 0.001
Clutch	2	13.1	2.1	0.136	3	23.5	16.7	< 0.001
Treatment*Clutch	2	41.8	6.6	0.002	3	0.5	0.4	0.666
Error	53	6.3			61	1.4		
Time	3	1654.7	749.3	< 0.001	6	415.8	867.7	< 0.001
Time*Treatment	3	91.2	41.3	< 0.001	6	5.6	11.6	< 0.001
Time*Clutch	6	7.9	3.6	0.002	18	4.9	10.3	< 0.001
Time*Treatment*Clutch	6	19.4	8.8	< 0.001	18	1.3	2.7	0.031
Error	159	2.2			366	0.5		

media (hereafter "high nutrient flies") did have higher nitrogen, lower C:N, lower lipid, higher protein and lower lipid:protein than flies reared on the potato flake medium alone (hereafter "low nutrient flies"; data in Schmidt et al. 2012).

Growth in the wandering spider, *Tigrosa helluo*.—Adult female *Tigrosa helluo* were collected in and around the agricultural fields at the Miami University Ecology Research Center (Oxford, Butler County, Ohio). Individuals were maintained in plastic containers (11 cm diameter × 8 cm high) with 1 cm of a peat/soil mix and fed two juvenile crickets (*Acheta domesticus*) twice per week. All females were fed similarly to reduce potential maternal effects. Three females produced egg sacs, which are carried attached to the female's spinnerets until they hatch. After hatching, spiderlings reside on the abdomen of the female for one to two weeks and then disperse (i.e., leave the mother's abdomen). For this experiment, I collected 20 dispersed spiderlings from each of three clutches produced by different females. The mass of dispersed spiderlings was 1.21 ± .02 mg (mean ± 1 SE). For this and the following experiment, spiderlings were separated before cannibalism occurred. A split-clutch design was used, in which spiderlings from each clutch were alternately assigned to high (n = 10) and low (n = 10) diet treatment groups. Each spiderling was placed in an individual translucent plastic container (8 cm diameter × 5 cm high) with one cm of moist peat moss. Individuals were fed *ad libitum* twice weekly with either low or high nutrient flies and weighed to the nearest 0.1 mg once weekly. The experiment was terminated after three weeks when individuals became too large to be maintained on *D. melanogaster*. Although I did not record the timing of molting, all individuals molted at least twiee during the experiment. At the end of the experiment, all individuals were sacrificed by freezing, and I measured carapace width, which is typically used as a measure of body size for wandering spiders (Jakob et al. 1996), to the nearest 0.01 mm using an ocular micrometer.

Growth in the web-building spider, *Pholcus phalangioides*.—Adult female *Pholcus phalangioides* with egg sacs were collected from a free-living population in Pearson Hall at Miami University. I collected similarly sized females with similarly sized egg sacs to reduce potential maternal effects. Egg sacs were removed from four separate individuals and allowed to hatch in separate deli containers (11 cm diameter × 8 cm high). For this experiment, I collected 20 dispersed spiderlings from each of the clutches and alternately assigned them to treatment groups. Each spiderling was placed in an individual translucent plastic container (3 cm diameter × 9 cm high). Individuals were fed ad libitum twice weekly with either low or high nutrient flies and weighed to the nearest 0.1 mg once weekly. The experiment was terminated after seven weeks when individuals became too large to be maintained on *D. melanogaster*. All individuals molted at least three times during the experiment. At the end of the experiment, all individuals were sacrificed by freezing, and I measured the combined length of the tibia and patella of one of the first pairs of legs, which is typically used as a measure of body size for web-building spiders (Jakob et al. 1996), to the nearest 0.01 mm using an ocular micrometer. In *P. phalangiodes*, tibia-patella length is highly correlated with carapace width (Schafer et al. 2008).

Statistical analyses.—I conducted repeated measures analysis of variance to test the effects of prey nutrient treatment, clutch and their interaction on mass separately for *T. helluo* and *P. phalangioides*. For measures of final body size, I conducted a two-factor analysis of variance to test the effects of prey nutrient treatment, clutch and their interaction on carapace width for *T. helluo*. I conducted a separate two-factor ANOVA to test the effects of the treatment factors on tibia-patella length for *P. phalangioides*. I used Tukey post hoc tests on least squares means for all possible comparisons to test for differences between low and high nutrient treatments of each clutch and for differences among clutches in growth on low or high nutrient treatments. Post-hoc comparisons were considered significant if $P < 0.05$. There were some deviations from normality and homogeneity of variance, although at least half of the treatment combinations for each species followed these assumptions. All statistical analyses were conducted in SAS 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Overall, there were significant effects of prey nutrient content on the growth of both *T. helluo* and *P. phalangioides* (Table 1, Fig. 1). Spiders raised on the high nutrient diet were, on average, 50% heavier than individuals on the low nutrient diet within three weeks for *T. helluo* and seven weeks for *P. phalangioides* (Fig. 1). Survival to the end of the experimental period was high in both *T. helluo* (overall: 98%) and *P. phalangioides* (overall: 86%).

Growth in the wandering spider, *Tigrosa helluo*.—There was a significant interaction between time, diet treatment and

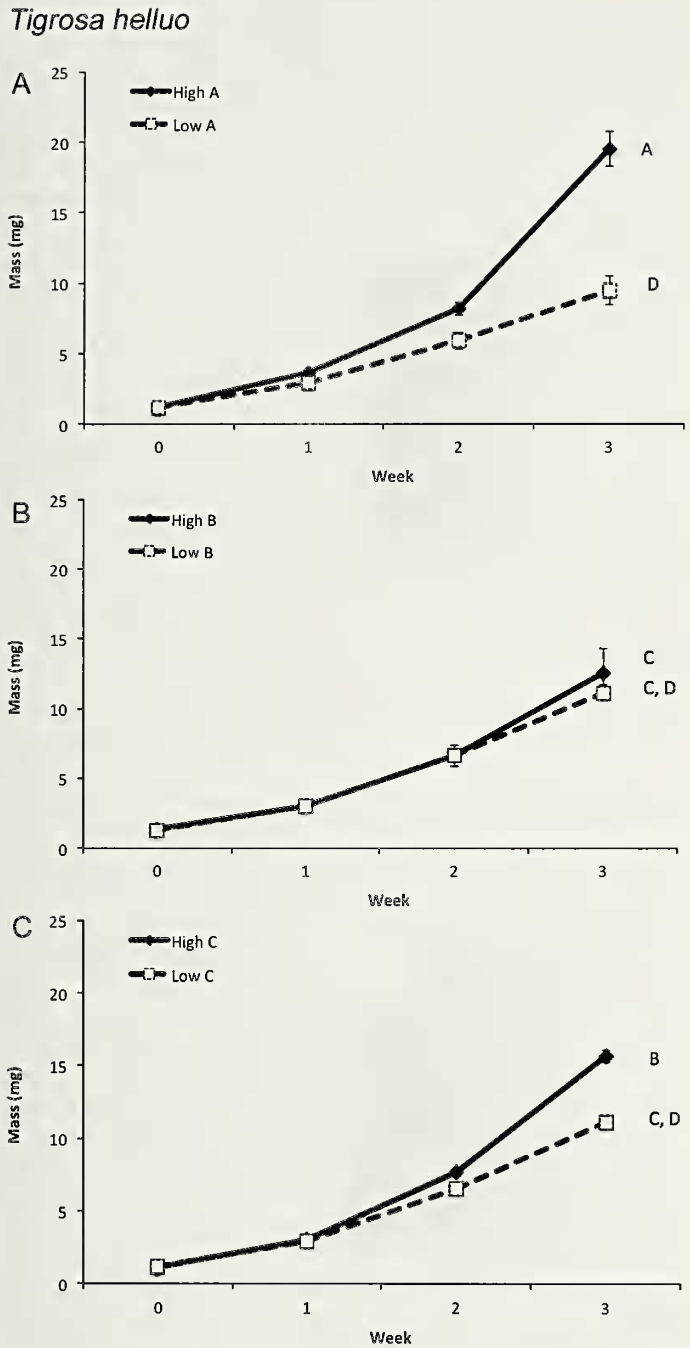

Figure 1.—Comparison of mean (± 1 SE) mass of juvenile *Tigrosa helluo* over three weeks on low or high nutrient diets from clutch A (A), clutch B (B), and clutch C (C). Post hoc comparisons were conducted for the final mass of spiders. Different letters show significant difference from each other at $\alpha = 0.05$.

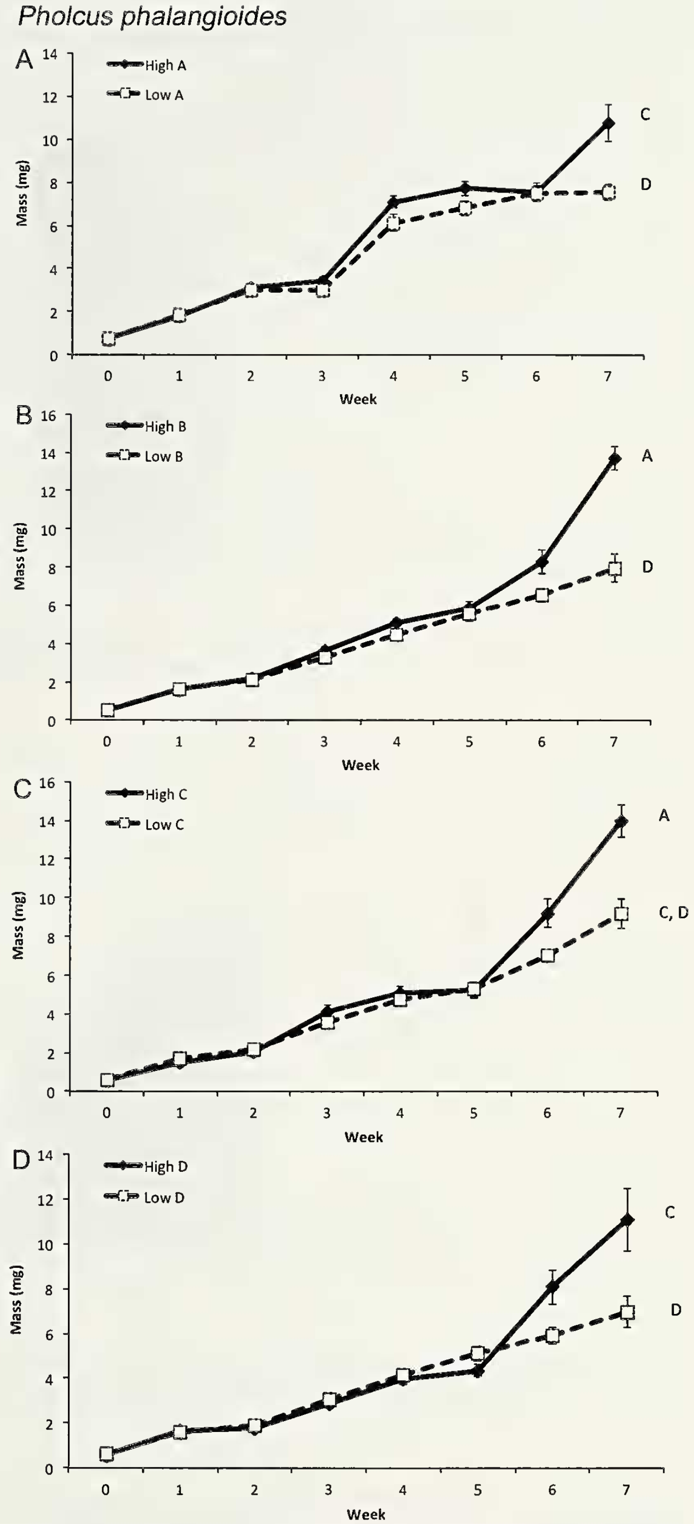

Figure 2.—Comparison of mean (± 1 SE) mass of juvenile *Pholcus phalangioides* over seven weeks on low or high nutrient diets from clutch A (A), clutch B (B), clutch C (C), clutch D (D). Post hoc comparisons were conducted for the final mass of spiders. Different letters show significant difference from each other at $\alpha = 0.05$.

clutch on the mass of juvenile *T. helluo*, indicating that the effects of prey nutrient content varied among clutches (Table 1, Fig. 1). Post hoc comparisons revealed significant differences between the mass of spiderlings on the low and high nutrient diets for clutches A and C, but no differences in the mass of spiderlings on the low and high nutrient diets for clutch B (Fig. 1).

There was a nonsignificant tendency for an interaction between clutch and diet on the final carapace width of *T. helluo* ($F_{2,48} = 3.02$, $P = 0.058$; Fig. 3A). As with mass, post hoc comparisons revealed that spiderlings from clutches A and C had significantly larger carapace widths on the high nutrient diet than on the low nutrient diet, but that there was no

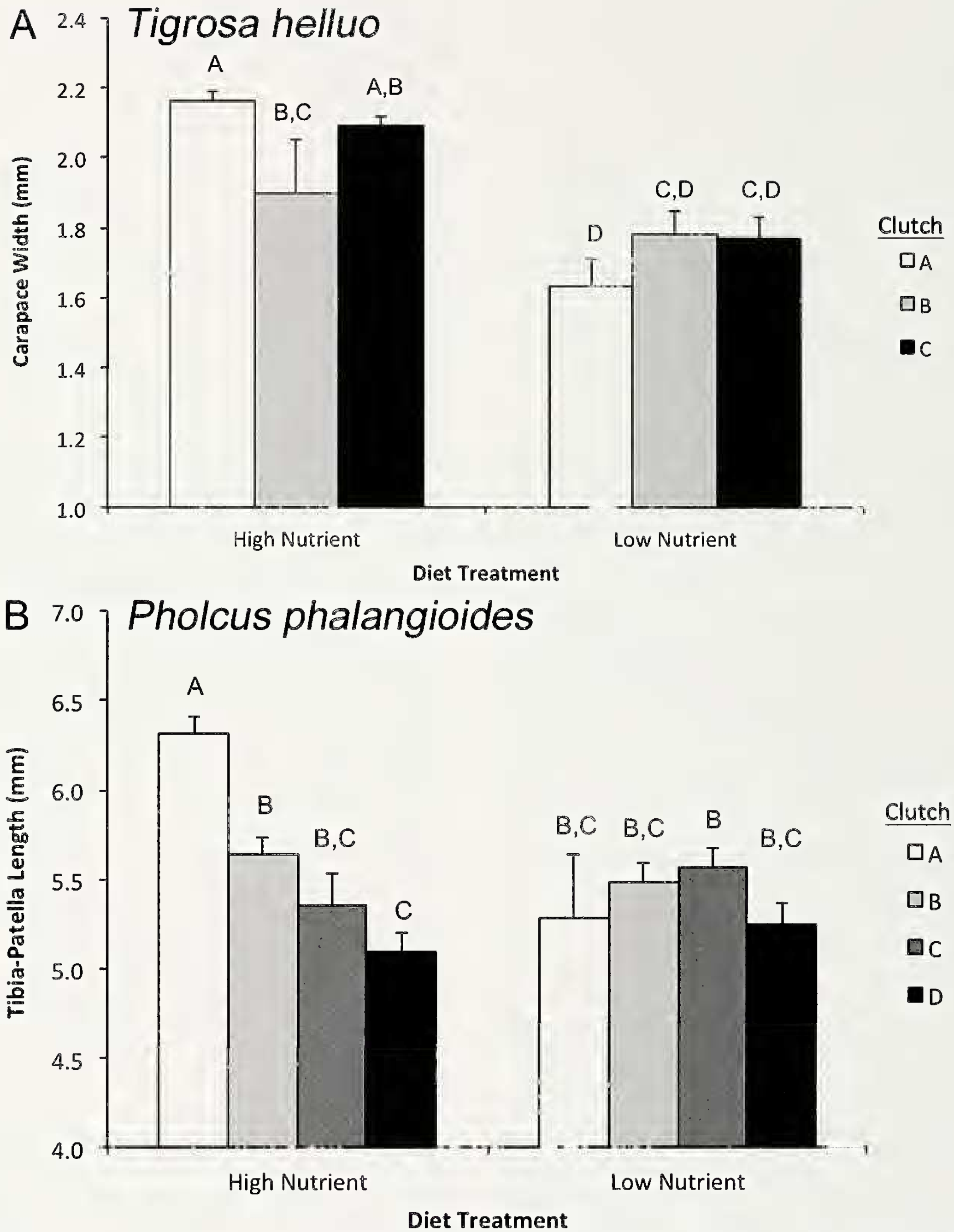

Figure 3.—Comparison of mean (± 1 SE) body size of A) *Tigrosa helluo* (measured as carapace width) and B) *Pholcus phalangioides* (measured as the length of the tibia and patella). Different letters show significant difference from each other at $\alpha = 0.05$.

significant effect of diet on the carapace width of clutch B (Fig. 3A).

Growth in the web-building spider, *Pholcus phalangioides*.—There was a significant interaction between time, diet treatment and clutch on the mass of juvenile *P. phalangioides*, indicating that the effects of prey nutrient content varied among clutches (Table 1, Fig. 1). In *P. phalangioides*, all four clutches showed higher mass on high nutrient diets (Fig. 2b). However, there were significant differences between clutches in the final mass of spiders on the high nutrient diet. Comparing spiderlings on the high nutrient diets, *P. phalangioides* from clutches B and C were significantly larger than those from clutches A and D (Fig. 2). There were no differences among clutches in the final mass of spiders on the low nutrient diet.

There was a significant interaction between diet treatment and clutch on the final tibia-patella length of *P. phalangioides* ($F_{3,59} = 5.59$, $P < 0.001$; Fig. 3B). Post hoc comparisons revealed a significantly larger tibia-patella length of spiderlings on the high nutrient diet than on the low nutrient diet for clutch A, but that clutches B, C, and D showed no significant effect of diet treatment on final tibia-patella length (Fig. 3B).

DISCUSSION

Many studies have demonstrated that the nutrient content of prey affects the survival and growth of spiders (Mayntz & Toft 2001; Mayntz et al. 2003; Jespersen & Toft 2003; Mayntz et al. 2005; Mayntz & Toft 2006). However, using a split-clutch design, my results demonstrate significant differences among clutches in the effects of prey nutrient content on both mass and body size of two spiders with different life history strategies. Some clutches showed large responses to prey nutrient content, while others showed no significant difference between individuals on the low and high diets. Depending upon the clutch, individuals on the high nutrient diet were 12–106% larger than individuals on the low nutrient diet (Fig. 2). Interestingly, there were no significant differences between clutches in the mass or body size of spiders on the low nutrient diet. The differences among clutches depended upon how large spiders grew when fed the high nutrient diet. The differences among clutches in the effects of diet were unexpected, given that individuals of both species were collected from relatively small geographic areas (*T. helluo* from one 13 ha field and *P. phalangioides* from one isolated population in a building). The large variation among clutches and qualitative differences in whether or not clutches significantly responded to diet treatment in this study highlight the importance of including this factor in studies of the effects of diet on survival and growth.

Clutch by diet interactions could have been a consequence of maternal or genetic effects (Mousseau and Dingle 1991). For maternal effects, adult females with different nutritional histories could influence the growth or survival of their offspring, depending upon the type or amount of nutrients provisioned to eggs (Mousseau & Dingle 1991; Bernardo 1996). The effect of maternal environment or condition on offspring performance has been demonstrated in a wide range of animals (Mousseau & Dingle 1991). For example, in spiders, sexual cannibalism of a small male by a female can affect the size, growth and survival of the subsequent offspring (Rabaneda-Bueno et al. 2008; Welke & Schneider 2012). More recent work has also highlighted the potential for epigenetic modification of gene expression in offspring, depending upon the conditions experienced by the mother (Bossdorf et al. 2008). However, the possibility of strong maternal effects in the current study was lessened by the fact that females used in this study were fed similarly before egg production.

Another explanation for these effects is that they were due to genetic variation in response to diet nutrient content. For example, in *Daphnia* spp. there is evidence that longer intergenic spacer (IGS) regions in the rDNA tandem repeat unit can result in higher rates of rRNA transcription and higher organismal growth rate (Elser et al. 2000; Weider et al. 2004). For example, Weider et al. (2005) demonstrated the existence of two clones of *Daphnia pulex* that differed in their response to food quality and the length of the IGS regions in their rDNA. When held under constant conditions in the laboratory, the long IGS region clone competitively excluded the short IGS region clone under conditions of high food quality, and the short IGS region clone excluded the long IGS region clone under conditions of low food quality (Weider et al. 2005). In spiders, Uhl et al. (2004) used a full-sibling design to demonstrate significant interactions between genotype and food quantity on the growth of juvenile *P. phalangiodes*. Similar full-sibling designs could be used to test for interactions between genotype and prey nutrient content using a wide range of nutrient manipulations.

Regardless of the mechanisms responsible for these effects, these results demonstrate significant differences among clutches in the effects of diet on spider growth, even among spiders collected from a limited geographic area. Future studies of spider nutrition should sample a wide range of individuals and explicitly incorporate clutch effects into the statistical design to 1) avoid potential spurious effects (i.e., due to a predominance of a particular genotype that is especially responsive or unresponsive), 2) capture the full range of variation in treatment effects, and 3) ensure that the results of studies are more generalizable.

ACKNOWLEDGMENTS

Many thanks to Chad Hoefler, David Mayntz, Ann Rypstra, Jason Schmidt and Kerri Wrinn for comments on a previous version of this manuscript. Funding was provided by the Graduate School and Department of Zoology at Miami University and NSF DBI 0216947 to A.L. Rypstra and DBI 0216776 to M.H. Persons.

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Manuscript received 3 October 2012, revised 18 January 2013.